

## Amendment and Response

Page 2 of 12

Serial No.: 10/673,538

Confirmation No.: 1846

Filed: September 29, 2003

For: METHODS AND KITS FOR THE DETECTION OF ERYTHROCYTES

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Amendments to the Claims

This listing of claims replaces all prior versions, and listings, of claims in the above-identified application:

Listing of Claims

1. (Currently Amended) A method for detecting occult blood in a specimen comprising:
  - (a) treating the specimen with a reacting solution comprising a strong reducing agent selected from the group consisting of sodium borohydride, potassium borohydride, calcium borohydride, copper borohydride, ammonium borohydride, benzyltriethylammonium borohydride, benzyl-triphenylphosphonium borohydride, bis (triphenylphosphine)copper(I) borohydride, cetyl-trimethylammonium borohydride, lithium borohydride, methytriocetylaminonium borohydride, tetramethylammonium borohydride, tetrabutylammonium borohydride, tetraethylammonium borohydride, lithium aluminum hydride, diborane, 9-BBN, dihydrogen, a grignard reagent, dialkylcopper lithium (lithium dialkylcuprate) reagents, sodium, alkyl sodium, and alkyl lithium; and
  - (b) monitoring the treated specimen for fluorescence, wherein fluorescence indicates the presence of occult blood, ~~and wherein the treated specimen fluoresces with a spectrum from about 530 nm to about 670 nm when excited at about 480 nm.~~
2. (Previously Presented) A method for detecting occult blood in a specimen comprising:
  - (a) treating the specimen with a reacting solution comprising a strong reducing agent; and
  - (b) monitoring the treated specimen for fluorescence;wherein fluorescence indicates the presence of occult blood; and wherein the strong reducing agent is sodium borohydride.
3. (Original) A method of claim 2, wherein the reacting solution is made up of approximately 0.1 percent to approximately 4 percent sodium borohydride.

## Amendment and Response

Page 3 of 12

Serial No.: 10/673,538

Confirmation No.: 1846

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For: METHODS AND KITS FOR THE DETECTION OF ERYTHROCYTES

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4. (Original) A method of claim 2, wherein the reacting solution is made up of approximately 0.2 percent sodium borohydride.
5. (Original) A method of claim 1, wherein the reacting solution is comprised primarily of phosphate buffered saline (PBS).
6. (Previously Presented) A method of claim 1, wherein the specimen is a biological specimen selected from the group consisting of feces, urine, cerebral spinal fluid, plural cavity fluid, thoracic cavity fluid and cerebral fluid.
7. (Previously Presented) A method of claim 1, wherein the fluorescence is monitored by a fluorescent spectrometer or a fluorescent microscope.
8. (Previously Presented) A method of claim 1, wherein the strong reducing agent is sodium borohydride.
9. (Currently Amended) A method for detecting one or more erythrocytes in a specimen, wherein the method comprises:
  - (a) treating the specimen with a strong reducing agent effective to enhance the fluorescence of any erythrocyte present in the specimen, said reducing agent selected from the group consisting of sodium borohydride, potassium borohydride, calcium borohydride, copper borohydride, ammonium borohydride, benxyltriethylammonium borohydride, benzyl-triphenylphosphonium borohydride, bis (triphenylphosphine)copper(I) borohydride, cetyl-trimethylammonium borohydride, lithium borohydride, methytrioctylammonium borohydride, tetramethylammonium borohydride, tetrabutylammonium borohydride, tetraethylammonium borohydride, lithium aluminum hydride, diborane, 9-BBN, dihydrogen, a grignard reagent, dialkylcopper lithium (lithium dialkylcuprate) reagents, sodium, alkyl sodium, and alkyl lithium; and
  - (b) monitoring the fluorescence emitted by the treated specimen, wherein fluorescence of

## Amendment and Response

Page 4 of 12

Serial No.: 10/673,538

Confirmation No.: 1846

Filed: September 29, 2003

For: METHODS AND KITS FOR THE DETECTION OF ERYTHROCYTES

---

one or more erythrocytes in the treated specimen indicates the presence of erythrocytes; and wherein the treated specimen fluoresces with a spectrum from about 530 nm to about 670 nm when excited at about 480 nm.

10. (Previously Presented) A method of claim 9, wherein the specimen is a biological specimen selected from the group consisting of feces, urine, cerebral spinal fluid, plural cavity fluid, thoracic cavity fluid and cerebral fluid.
11. (Original) A method of claim 9, wherein the erythrocytes are monitored by a fluorescent microscope.
12. (Currently Amended) A method for quantifying the amount of occult blood in a specimen comprising:
  - (a) exposing the specimen to a reacting solution comprising a strong reducing agent selected from the group consisting of sodium borohydride, potassium borohydride, calcium borohydride, copper borohydride, ammonium borohydride, benxyltriethylammonium borohydride, benzyl-triphenylphosphonium borohydride, bis (triphenylphosphine)copper(I) borohydride, cetyl-trimethylammonium borohydride, lithium borohydride, methytriocetylammmonium borohydride, tetramethylammmonium borohydride, tetrabutylammmonium borohydride, tetraethylammmonium borohydride, lithium aluminum hydride, diborane, 9-BBN, dihydrogen, a grignard reagent, dialkylcopper lithium (lithium dialkylcuprate) reagents, sodium, alkyl sodium, and alkyl lithium; and
  - (b) monitoring the exposed specimen for fluorescence, wherein fluorescence indicates the amount of occult blood present in the specimen; and ~~wherein the exposed specimen fluoresces with a spectrum from about 530 nm to about 670 nm when excited at about 480 nm.~~

## Amendment and Response

Page 5 of 12

Serial No.: 10/673,538

Confirmation No.: 1846

Filed: September 29, 2003

For: METHODS AND KITS FOR THE DETECTION OF ERYTHROCYTES

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13. (Previously Presented) A method of claim 1 wherein the specimen is a fecal specimen, the method further comprising, prior to step (a), purifying the fecal specimen to substantially remove all materials that will interfere with measuring the fluorescence of the fecal specimen.

14.-25. (Canceled)

26. (Currently Amended) A method for determining whether a subject is at risk of developing, or suffers from, a disease associated with occult blood the method comprising:

- (a) treating a specimen obtained from the subject with a reacting solution comprising a strong reducing agent selected from the group consisting of sodium borohydride, potassium borohydride, calcium borohydride, copper borohydride, ammonium borohydride, benzyldiethylammonium borohydride, benzyl-triphenylphosphonium borohydride, bis (triphenylphosphine)copper(I) borohydride, cetyl-trimethylammonium borohydride, lithium borohydride, methyloctylammonium borohydride, tetramethylammonium borohydride, tetrabutylammonium borohydride, tetraethylammonium borohydride, lithium aluminum hydride, diborane, 9-BBN, dihydrogen, a grignard reagent, dialkylcopper lithium (lithium dialkylcuprate) reagents, sodium, alkyl sodium, and alkyl lithium; and
- (b) monitoring the treated specimen for fluorescence, wherein fluorescence indicates the presence of occult blood and the likelihood that the subject may develop or has developed the disease; ~~and wherein the treated specimen fluoresces with a spectrum from about 530 nm to about 670 nm when excited at about 480 nm.~~

27. (Original) A method of claim 26, wherein the disease is gastrointestinal tumors, kidney tumors, bladder tumors, lung cancer, thoracic wall cancer, or parasite infestation and the subject is a human.

28. (Original) A method of claim 26, wherein the specimen is a biological specimen selected

## Amendment and Response

Page 6 of 12

Serial No.: 10/673,538

Confirmation No.: 1846

Filed: September 29, 2003

For: METHODS AND KITS FOR THE DETECTION OF ERYTHROCYTES

from the group consisting of feces, urine, cerebral spinal fluid, plural cavity fluid, thoracic cavity fluid and cerebral fluid.

29. (Currently Amended) A method for determining the extent and spatial distribution of erythrocytes trapped in cerebral tissues microvasculature comprising:

- (a) treating cerebral tissue microvasculature with a reacting solution comprising a strong reducing agent selected from the group consisting of sodium borohydride, potassium borohydride, calcium borohydride, copper borohydride, ammonium borohydride, benxyltriethylammonium borohydride, benzyl-triphenylphosphonium borohydride, bis (triphenylphosphine)copper(I) borohydride, cetyl-trimethylammonium borohydride, lithium borohydride, methytriocetyl ammonium borohydride, tetramethylammonium borohydride, tetrabutylammonium borohydride, tetraethylammonium borohydride, lithium aluminum hydride, diborane, 9-BBN, dihydrogen, a grignard reagent, dialkylcopper lithium (lithium dialkylcuprate) reagents, sodium, alkyl sodium, and alkyl lithium; and
- (b) monitoring the treated tissue for fluorescence; wherein the fluorescence indicates the extent and spatial distribution of erythrocytes trapped in cerebral tissue microvasculature.

30. (Previously Presented) A method of claim 29, wherein the vasculature is flushed with heparinized saline by cardiac perfusion to remove erythrocytes from functional post-ischemic brain microcirculation prior to treatment with the reacting solution.

31. (Canceled)

32. (Currently Amended) A method for determining whether a subject is at risk of developing, or suffers from, cerebral vascular trauma or bleeding, the method comprising:

- (a) treating cerebral tissue microvasculature in situ or ex vivo with a reacting solution comprising a strong reducing agent selected from the group consisting of sodium borohydride, potassium borohydride, calcium borohydride, copper borohydride, ammonium borohydride,

## Amendment and Response

Page 7 of 12

Serial No.: 10/673,538

Confirmation No.: 1846

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For: METHODS AND KITS FOR THE DETECTION OF ERYTHROCYTES

benxyltriethylammonium borohydride, benzyl-triphenylphosphonium borohydride, bis (triphenylphosphine)copper(I) borohydride, cetyl-trimethylammonium borohydride, lithium borohydride, methytrioctylammonium borohydride, tetramethylammonium borohydride, tetrabutylammonium borohydride, tetraethylammonium borohydride, lithium aluminum hydride, diborane, 9-BBN, dihydrogen, a grignard reagent, dialkylcopper lithium (lithium dialkylcuprate) reagents, sodium, alkyl sodium, and alkyl lithium; and

(b) monitoring the treated tissue for fluorescence;

wherein the fluorescence indicates the extent and spatial distribution of erythrocytes trapped in cerebral tissue microvasculature which is indicative of the likelihood that the subject may develop or has developed cerebral vascular trauma or bleeding.

33. (Currently Amended) A method of detecting the presence or past existence of erythrocytes in a specimen or sample comprising:

(a) treating the specimen or sample with a reacting solution comprising a strong reducing agent selected from the group consisting of sodium borohydride, potassium borohydride, calcium borohydride, copper borohydride, ammonium borohydride, benxyltriethylammonium borohydride, benzyl-triphenylphosphonium borohydride, bis (triphenylphosphine)copper(I) borohydride, cetyl-trimethylammonium borohydride, lithium borohydride, methytrioctylammonium borohydride, tetramethylammonium borohydride, tetrabutylammonium borohydride, tetraethylammonium borohydride, lithium aluminum hydride, diborane, 9-BBN, dihydrogen, a grignard reagent, dialkylcopper lithium (lithium dialkylcuprate) reagents, sodium, alkyl sodium, and alkyl lithium; and

(b) monitoring the treated specimen or sample for fluorescence, wherein fluorescence indicates the presence or past existence of erythrocytes in the sample or specimen; and wherein the treated specimen fluoresces with a spectrum from about 530 nm to about 670 nm when excited at about 480 nm.

34. (Canceled)

**Amendment and Response**

Page 8 of 12

Serial No.: 10/673,538

Confirmation No.: 1846

Filed: September 29, 2003

For: METHODS AND KITS FOR THE DETECTION OF ERYTHROCYTES

35. (Currently Amended) A method of claim 1 wherein the strong reducing agent is selected from the group consisting of sodium borohydride, potassium borohydride, calcium borohydride, copper borohydride, ammonium borohydride, benzyltriethylammonium borohydride, benzyltriphenylphosphonium borohydride, bis (triphenylphosphine)copper(I) borohydride, cetyltrimethylammonium borohydride, lithium borohydride, methyltriethylammonium borohydride, tetramethylammonium borohydride, tetrabutylammonium borohydride, tetraethylammonium borohydride, lithium aluminum hydride, diborane, 9-BBN, dihydrogen, a grignard reagent, dialkylcopper-lithium (lithium dialkylcuprate) reagents, sodium, alkyl sodium, and alkyl lithium; wherein the treated specimen fluoresces with a spectrum from about 530 nm to about 670 nm when excited at about 480 nm.